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Alpha-1 Antitrypsin Deficiency – A Genetic Risk Factor for COPD

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1. Introduction

Alpha-1 antitrypsin deficiency (AATD) is a hereditary disorder characterised by low circulating levels of the key antiprotease alpha-1 antitrypsin (AAT) and is associated with the development of chronic obstructive pulmonary disease (COPD), often by the 3rd or 4th decade, and liver disease. The two most common SERPINA1 mutations associated with AATD are the Z and S mutations, and the vast majority of AATD individuals diagnosed with COPD are ZZ homozygotes. AATD is an under-diagnosed condition with the majority of cases misdiagnosed as COPD. The World Health Organisation (WHO) and the American Thoracic Society/European Respiratory Society (ATS/ERS) advocate a targeted screening approach for the detection of AATD in patients with COPD, non-responsive asthma, cryptogenic liver disease and first degree relatives of known AATD patients (Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting 1997; American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). It is our contention that a diagnosis of AATD gives the clinician a vital and unique opportunity for early medical intervention and the possible prevention of COPD in both the affected individual and first-degree relatives. Unfortunately, despite huge strides in awareness and understanding of this condition, this opportunity is too often missed.

2. Alpha-1 antitrypsin deficiency (AATD)

2.1 Alpha-1 antitrypsin (AAT)

AAT is a secretory glycoprotein produced by the liver and is the most abundant serum antiprotease in circulation (Kueppers 1971). While the majority of AAT in the body is hepatocyte-derived, it is actively transcribed and secreted by other cell types including monocytes (Carroll et al. 2010), macrophages (Mornex et al. 1986), neutrophils (Bergin et al. 2010), intestinal epithelial cells (Perlmutter et al. 1989), and various epithelial cells in the lung (Hu and Perlmutter 2002; Venembre et al. 1994; Cichy, Potempa, and Travis 1997), albeit in smaller quantities. In keeping with its role as an acute phase reactant, the

hepatocyte expresses approximately 200 times more AAT mRNA than other cells (Rogers et al. 1983) and serum levels can rapidly increase by between two- and five-fold during infection, trauma, surgery and burns (Kossmann et al. 1995; Voulgari et al. 1982; Sandford et al. 1999; Jeschke, Barrow, and Herndon 2004). The AAT released by inflammatory cells is more relevant to the local inflammatory milieu, serving to limit local degradation of the extracellular matrix by proteases released from migrating immune cells (Knoell et al. 1998). In a similar fashion, the range of cells producing AAT locally in the lung underlines its key role in protecting the fragile lung parenchyma against proteolytic damage. AAT was originally named because of its ability to inhibit pancreatic trypsin (Schultze, Heide, and Haupt 1962) and while its principal function as an antiprotease has been well established, AAT has significant anti-inflammatory properties affecting numerous cell types, and has been implicated in areas as diverse as apoptosis (Petrache, Fijalkowska, Medler, et al. 2006), chemotaxis (Bergin et al. 2010), tissue repair (Dabbagh et al. 2001), innate immunity (Bergin et al. 2010), and cell signalling (Koulmanda et al. 2008).

The gene encoding AAT is the SERPINA1 gene, found on the long arm of chromosome 14 at q32.1 and is composed of four coding exons (II, III, IV, V), three untranslated exons (Ia, Ib, and Ic) in the 5′ region and six introns. The hepatocyte transcription start site resides within exon Ic, while the mononuclear phagocyte transcription start sites reside within exons Ia and Ib. The SERPINA1 gene encodes a 418 amino acid protein, which includes a 24 amino acid signal peptide. The mature form of the protein is a 394 amino acid, 52 kDa glycoprotein with three asparagine-linked carbohydrate side chains (Carrell et al. 1982). Serum concentrations of AAT are genetically determined by the two alleles of the SERPINA1 coding gene, expressed co-dominantly, and normal serum concentrations of AAT are in the range of 1.04 g/L to 2.76 g/L (20 – 53 μM) (Brantly et al. 1991). Over 30 mg/kg body weight of AAT is secreted into the circulation daily, and the glycosylated protein has a serum half-life of 4 – 5 days (Jeppsson et al. 1978; Jones et al. 1978).
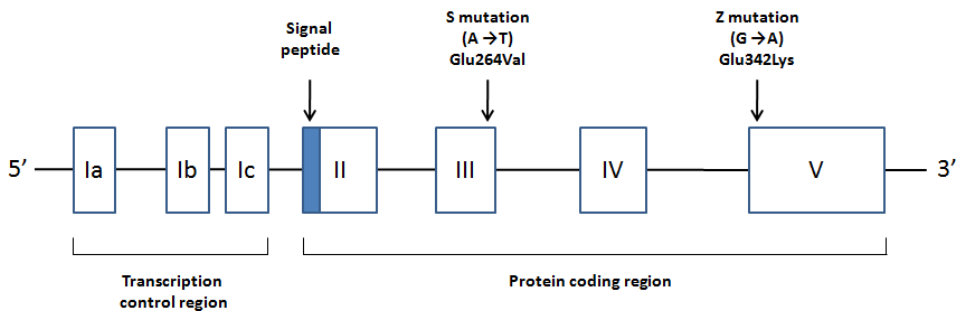

Fig. 1. SERPINA1 gene encoding the alpha-1 antitrypsin protein. The protein coding region is within exons II-V, with the S and Z mutations arising in exon III and exon V respectively.

Although AAT diffuses though all tissues of the body its main site of action is the lung, where it protects the fragile alveolar, connective, and epithelial tissues from proteolytic attack by the omnivorous serine protease neutrophil elastase (NE) (Bieth 1986). In this regard, AAT is the archetype of the serine protease inhibitor or serpin super-family, whose primary function is to inhibit a family of proteolytic enzymes with serine at the active site.

Other prominent serpins include alpha-1 antichymotrypsin, alpha-2 antiplasmin, antithrombin, plasminogen activator inhibitor I, and C1 inhibitor (Law et al. 2006). The serpins act like molecular snares or mousetraps, existing in a metastable state, until they entice the unsuspecting cognate protease into their trap, and their energy is released by cleavage of their reactive centre loop (Carrell and Lomas 2002). AAT irreversibly inhibits serine proteases by presenting its inhibitory amino acid sequence as a mobile, exposed reactive centre loop with a methionine residue at its centre (Elliott et al. 1996). This reactive centre loop is a pseudosubstrate for target proteases, such as NE, and upon docking of the protease AAT undergoes a conformational change that results in the irreversible inhibition and destruction of the protease (Huntington, Read, and Carrell 2000). Although AAT can regulate the activity of many other serine proteases including trypsin, chymotrypsin, plasmin, thrombin, plasminogen, cathepsin G and proteinase 3 (Silverman et al. 2001; Travis and Salvesen 1983), it exhibits the highest association constant for NE which is 25 times greater than for any other protease (Beatty, Bieth, and Travis 1980). In addition, NE is the most relevant protease in the lung setting as it is released in large amounts by activated neutrophils (Gadek 1992).

2.2 Alpha-1 antitrypsin deficiency (AATD)

2.2.1 History and clinical features

AATD is a hereditary monogenic disorder caused by mutations within the SERPINA1 gene, previously known as the "protease inhibitor" or "PI" gene. The condition was first reported in the early 1960s by Carl-Bertil Laurell and Sten Eriksson at the University of Lund in Sweden when they noticed the absence of the alpha-1 globulin band on routine serum protein electrophoresis of five patients (Laurell and Eriksson 1963). It was already known that over 90% of the alpha-1 band comprised a single protein that was capable of inhibiting the proteolytic enzyme trypsin (Jacobsson 1955; Schultze, Heide, and Haupt 1962). Following a review of the five patients, three were found to have developed "a degenerative pulmonary disease" at a young age and the condition we now know as AATD was born (Eriksson 1964). Interestingly, the oldest reported case is thought to have been a young girl who had remained undiscovered in the Alaskan permafrost for over 800 years (Zimmerman, Jensen, and Sheehan 2000). More recently there has been speculation that the composer Frédéric Chopin suffered from AATD with his physician failing to confirm pulmonary tuberculosis instead discovering "a disease not previously encountered" on autopsy (Kuzemko 1994; Kubba and Young 1998).

AATD is associated with a substantially increased risk for the development of COPD, often by the third or fourth decade, and is also associated with risks for development of liver disease (Sharp et al. 1969; Lieberman, Mittman, and Gordon 1972; Sveger 1976), cutaneous panniculitis (Edmonds, Hodge, and Rietschel 1991), bronchiectasis (King et al. 1996), vasculitis (Lewis et al. 1985), and Wegener's granulomatosis (Barnett, Sekosan, and Khurshid 1999). AATD is characterised by misfolding of the AAT protein and belongs to a class of genetic diseases underpinned by aberrant protein folding collectively termed conformational disorders (Gooptu and Lomas 2009; Greene et al. 2008). In addition, AATD is a paradigm of the subclass of conformational diseases known as the serpinopathies (Carrell and Lomas 1997). Serpins have a highly conserved structure of three β–sheets, nine α–helices, and an exposed reactive centre loop (Gettins 2002). Serpinopathies are
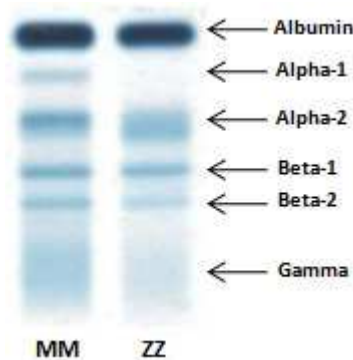
Fig. 2. Serum protein electrophoresis comparison between MM and ZZ individuals. Note the complete absence of the alpha-1 globulin band in ZZ individual as first described by Laurell and Eriksson in 1963.

characterized by mutations that subvert this serpin primary structure to permit the formation of intracellular polymers and this can have pronounced deleterious consequences. In the rare condition familial encephalopathy with neuroserpin inclusion bodies (FENIB) polymerisation of the neuron-specific serpin neuroserpin, caused by point mutations in the shutter domain region of the protein, favours the accumulation of intraneuronal aggregates and eventually leads to early onset dementia (Davis et al. 1999; Belorgey et al. 2002). In contrast to this gain of (pathological) function causing dementia, a loss of (physiological) function, specifically a lack of circulating serpin in individuals with C1 inhibitor, antithrombin, and alpha-1 antichymotrypsin deficiency facilitates unchecked activity of cognate proteases and the development of angioedema, thrombosis, and COPD, respectively (Lomas and Mahadeva 2002).

2.2.2 Molecular pathophysiology

The SERPINA1 gene is highly pleiomorphic with over 120 allelic variants, including 80 deficiency alleles, identified to date (DeMeo and Silverman 2004). The majority of individuals carry two copies of the normal AAT allele, termed M, and are designated M homozygous (Fagerhol and Laurell 1970). The technique of starch gel electrophoresis originally used to separate AAT variants is responsible for the nomenclature used to identify the earliest described variants. These variants were originally designated according to how fast they migrated on starch gel, for example M (medium), S (slow), and F (fast) (Fagerhol and Laurell 1970). As detection techniques advanced and proteins began to be separated on the basis of their isoelectric point (for AAT between pH 4 – 5), the nomenclature system was revised so AAT variants were designated with earlier letters of the alphabet if exhibiting anodal migration and later letters of the alphabet if exhibiting cathodal migration. Furthermore, as the letters of the alphabet were exhausted, place of origin names are used in addition to the letter of the closest anodal allele (Cox, Johnson, and Fagerhol 1980). More precisely the birthplace of the first described individual to carry a novel allele is used, for example Q0cairo was used to describe a novel Null mutation found in the first recognised case whose birthplace was Cairo (Zorzetto et al. 2005).

Mutations in the SERPINA1 gene that confer an increased risk for the development of COPD and/or liver disease are those in which deficiency or Null alleles are combined in homozygous or heterozygous states, and encode AAT plasma levels below a putative protective threshold of 11 μM or 0.57 g/L (Brantly et al. 1991). The two most common mutations associated with disease in populations of European descent are the Z (Glu342Lys) and S (Glu264Val) mutations, and both are caused by a single amino acid replacement of glutamic acid at positions 342 and 264 of the mature protein, respectively. In general, AAT alleles can be classified according to plasma levels and function and divided among four broad groups:

a.   Normal

Normal alleles are most commonly M subtypes which account for 95% of gene variants and are characterized by normal plasma levels in homozygotes (greater than 1.04 g/L or 20 μM). Other normal variants include V, T, and Lfrankfurt (Faber et al. 1994).

b.   Deficient

The Z allele is the most common deficiency variant, with plasma levels of ZZ homozygotes in the range of 0.10 – 0.52 g/L (2 – 10 μM). The S variant is also common but is only clinically significant if inherited with Z or other severe mutations (Mmalton, Null etc.). For example, SZ individuals have AAT plasma levels in the range of 0.33 – 0.98 g/L (6 - 20 μM).

c.   Null

The class of SERPINA1 mutations termed silent or "Null" cause a complete abrogation of AAT production (Lee and Brantly 2000) and while ultra rare, confer a particularly high risk of emphysema (Fregonese et al. 2008). As these mutations do not cause the AAT protein to polymerise they pose no risk of liver disease. Most frequent among this class are those mutations that introduce a premature stop codon, for example Q0cairo (Zorzetto et al. 2005).

d.   Dysfunctional

Like Null variants, dysfunctional alleles are extremely rare. For example, the single amino acid change caused by the Pittsburgh mutation (Met358Arg) at the active site of the AAT molecule converts it from an elastase inhibitor to a thrombin inhibitor and was discovered in a child who died from an episodic bleeding disorder (Owen et al. 1983).

The Z mutation (Glu342Lys) in AAT is an excellent example of the destabilizing effect of a mutation near the critical reactive centre loop of the protein. The proximity of the Z mutation to the loop and its location at the head of strand 5 of β–sheet A causes the AAT molecule to adopt a more open and promiscuous conformation (Gooptu et al. 2000). Thus, the β–sheet A of one molecule can readily accept the reactive loop of another AAT molecule to form a dimer. Elongation of this dimer leads to the formation of loop-sheet polymers in which the reactive centre loop of one AAT molecule sequentially inserts into the accessible β–sheet of another (Lomas et al. 1992; Sivasothy et al. 2000; Ekeowa et al. 2010). This mechanism of polymerization forms the basis for the liver disease observed in ZZ individuals, and the plasma deficiency arising from the intracellular accumulation of entangled polymers of AAT in the liver forms the basis for the lung disease observed in ZZ individuals. The Siiyama (Ser53Phe) and Mmalton (deletion of 52Phe) alleles predispose to polymerisation of the AAT molecule through a similar mechanism of loop-sheet formation.

Variant	Mutation	Effect	Disease Risk
Z	$\underline{G}$AG – $\underline{A}$AG, Glu342Lys	Polymerisation, impaired secretion and severe plasma deficiency	Lung & liver
Siiyama	T$\underline{C}$C – T$\underline{T}$C, Ser53Phe	Polymerisation, impaired secretion and severe plasma deficiency	Lung & liver
Mmalton	ΔTTC, ΔPhe52	Polymerisation, impaired secretion and severe plasma deficiency	Lung & liver
Null	Mutations causing gene deletion, premature stop codon or mRNA degradation	No AAT production	Lung
S	G$\underline{A}$A – G$\underline{T}$A, Glu264Val	Impaired secretion and mild plasma deficiency	Lung & liver (in compound heterozygotes e.g. SZ)
I	$\underline{C}$GC – $\underline{T}$GC, Arg39Cys	Impaired secretion and mild plasma deficiency	Lung & liver (case reports in compound heterozygotes e.g. IZ)
F	$\underline{C}$GT – $\underline{T}$GT, Arg223Cys	Defective neutrophil elastase inhibition	Lung (case reports in compound heterozygotes e.g. FZ)

Table 1. Molecular basis of the most common SERPINA1 variants associated with disease.
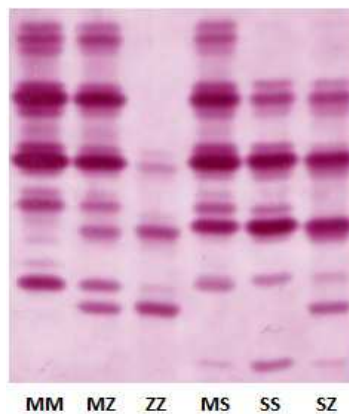

MM   MZ   ZZ   MS   SS   SZ

Fig. 3. Phenotype analysis of the most common deficient AAT variants by agarose gel isoelectric focusing followed by immunofixation (Sebia).

The Siiyama allele is the most common cause of AATD in Japan (Seyama et al. 1995), while the Mmalton variant (also known as Mcagliari and Mnichinan) is the most commonly found allele in Sardinia (Ferrarotti et al. 2005).

The formation of rogue AAT polymers can also be caused by the S (Glu264Val) and I (Arg39Cys) alleles, which are associated with milder plasma deficiencies and less risk of disease (Curiel et al. 1989; Graham et al. 1989). The point mutations underlying these variants cause less disruption to β–sheet A when compared to the Z mutation. Thus, the rate of polymer production for each variant is much slower than Z AAT, leading to less retention of protein within liver cells, milder plasma deficiency, and a negligible risk of disease in heterozygotes. However, there is a risk of disease in compound heterozygotes. If a mild, slowly polymerising I or S variant of AAT is inherited with a rapidly polymerising Z (or potentially Mmalton) variant, the two variants when co-expressed can interact to form heteropolymers within hepatocytes, leading to cirrhosis and plasma deficiency (Mahadeva et al. 1999). In addition to the pathophysiological effects of polymer formation and plasma deficiency, the Z AAT protein that does make it out of the liver and is available to defend the lungs is a poor inhibitor of NE. It has been shown that Z AAT is five times less effective at inhibiting NE when compared to normal M AAT (Ogushi et al. 1987). The S AAT protein is also a slightly less efficient inhibitor of NE when compared to M AAT, but the reduction is marginal in contrast to Z AAT (Ogushi et al. 1988).

The final deficient mutation that warrants mention is the F (Arg223Cys) allele. Like the Z, S, and I variants, the anodal F variant was first described by starch gel electrophoresis (Fagerhol and Braend 1965) but the molecular basis for the allele was not identified until much later (Okayama et al. 1991). The point mutation in this variant introduces a cysteine instead of an arginine, and the same amino acid substitution occurs in the I variant. The normal AAT molecule possesses a single cysteine residue and the introduction of a second cysteine potentially favours the formation of disulphide bonds intramolecularly and intermolecularly with other AAT molecules. Interestingly, and possibly a reflection of the extra cysteine residue, the major F bands run as doublets on IEF gels. In the disease context, the inhibitory activity of the F AAT protein against NE is reduced, with the *in vivo* inhibition time for FZ three times longer than for MZ (Cook et al. 1996). This would suggest that individuals who co-inherit the F allele with another severe deficiency allele such as Z or Null would have a high risk for the development of COPD. The rate of polymerisation of the F variant has not been investigated but it may well exhibit a higher rate of polymerisation. A case report described finding hepatomegaly and globules positive for AAT in a liver biopsy from an FZ individual with emphysema (Kelly et al. 1989). Unfortunately, there have been no reports published to date describing F homozygotes (or I homozygotes) as these might shed some light on the polymerigenicity of the F protein and associated risk of disease.

2.2.3 Epidemiology

Since the first description of AATD as a distinct clinical entity by Laurell and Eriksson almost 50 years ago, a wealth of data pertaining to the epidemiology of AATD has been accumulated. However, although a large number of groups have been investigated, the majority of studies have been biased to varying degrees as they investigated cohorts of symptomatic individuals, blood donors, soldiers, seamen, hospital staff, pregnant women, rheumatic disorders or other diseases, newborn infants and work-based employees (Blanco,
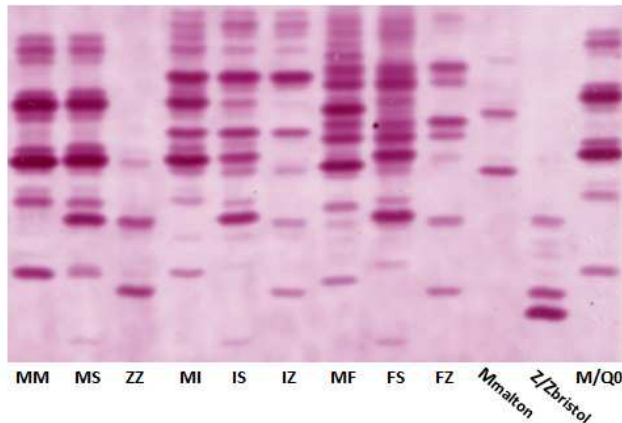
Fig. 4. Phenotype analysis of rare deficient AAT variants (I, F, Mmalton, Zbristol, and Q0) by isoelectric focusing followed by immunofixation. Reference MM, MS, and ZZ standards are included for clarity. The Mmalton variant depicted is from an individual homozygous for this mutation.

Fernandez, and Bustillo 2001). Allele frequencies collected from patients with pulmonary disease cannot be included as they would yield disproportionately high allele frequencies. Findings from several investigations of healthy control cohorts are also unreliable as these by their definition exclude symptomatic ZZ, SZ, and MZ individuals, and this leads to underestimation of the allele frequencies in a general population. Studies of blood bank donors also risk underestimating AATD as these donors tend to be healthy individuals. To our knowledge, a survey carried out in Asturias (Spain) is the only truly representative investigation of AAT allele frequencies in a general population as the subjects were of all ages and randomly selected from a municipal population register (Blanco et al. 1999).

The small sample size and the less accurate isoelectrofocusing methods employed in many of these population studies represent significant limitations. To illustrate this point, a small study was performed in Ireland in 1992 to determine if an association existed between AATD and coeliac disease (Bourke et al. 1993). AAT phenotypes in 111 coeliacs were compared to those in 250 blood donor controls including some hospital staff. Allele frequencies for Z and S in the blood donor group were determined to be 0.008 and 0.04, respectively, using a polyacrylamide gel (pH 4 – 5) isoelectric focusing method. We subsequently carried out a larger analysis of 1,100 individuals randomly selected from the national electoral register and genotyped this group for Z and S mutations. In contrast to the 1983 data, we found allele frequencies for Z and S to be 0.022 and 0.054, respectively (Carroll et al. 2011). The reason for this disparity is most probably due to the small sample size of the original cohort. Methodological limitations may also be a factor. We have previously employed the same phenotyping method (Pharmacia) as was used in the 1983 study and found the MZ phenotype was often difficult to correctly identify and was uncomfortably similar to the M2 subtype, compared to the more accurate and specific Sebia method used in the more recent study. Similar to the revised findings on the prevalence of AATD in Ireland, many of the studies in other countries may have significantly underestimated the frequency of the Z and S mutations due to small sample size and/or methodological limitations.

Moreover, as current genotyping methods only identify Z and S alleles it is worth considering that allele frequencies described in population studies using genotyping methods will potentially underestimate AATD as the rarer SERPINA1 mutations such as I, F, and Mmalton are missed by genotyping methods.

Across Europe the frequency of the Z and S mutations varies widely between countries, geographic regions, and ethnic groupings. It is estimated that approximately 3 - 4% of northern Europeans carry the Z allele and 6% carry the S allele (Blanco, de Serres et al. 2006). The frequency of the Z variant is highest in northern and western European countries with a mean gene frequency of 0.014, peaking in southern Scandinavia with a gene frequency of greater than 0.02, and in general declines from west to east and north to south throughout the continent (Luisetti and Seersholm 2004). The highest frequency for the Z allele recorded to date of 0.045 was found in western Latvia in a region that has seen centuries of immigration from mainland Sweden (Beckman et al. 1999). Haplotype analysis has estimated that the Z mutation first arose from a single origin 66 generations or 2,000 years ago (Cox, Woo, and Mansfield 1985; Byth, Billingsley, and Cox 1994) and its high frequency in southern Scandinavia suggests that the mutation arose in this area and was subsequently dispersed by migration patterns, for example the Viking colonisation of north-western Europe between 800 and 1200 AD (Hutchison 1998).

The region with the highest frequency of the S allele described to date is the Iberian Peninsula with a mean gene frequency of 0.0564. The highest reported frequencies of the S allele in Europe are in the region of Galicia in north-western Spain with 149 alleles found per 1000 (0.149) (Carracedo and Concheiro 1983) and in a Portuguese study with 152 alleles found per 1000 (0.152) (Santos Rosa and Robalo Cordeiro 1986). The allele frequency for S appears to decrease along south to north and west to east gradients, and is extremely low in Serbia and Russia (Blanco, Fernandez, and Bustillo 2001). In general high S frequencies are found all along the western Atlantic seaboard but accepting the premise that the higher the allele frequency in a particular country, the more likely it is to have arisen there first, it would appear the S allele arose in the north-western corner of the Iberian peninsula between 10,000 and 15,000 years ago (Seixas et al. 2001).

The only other AAT alleles whose origins can be reliably predicted are the Siiyama mutation, which appears to have arisen exclusively in Japan, and the Mmalton mutation, which is remarkably common in central Italy and on the island of Sardinia (Ferrarotti et al. 2005). Interestingly, the same mutation underlying Mmalton appears to have also arisen independently in the Japanese population (Matsunaga et al. 1990). Undoubtedly, more surveys relating to the epidemiology of AATD are necessary as there is a remarkable absence of AAT allele frequency data in many regions of Europe, particularly in eastern countries, as well as the lack of data pertaining to the frequencies and origins of rarer AAT alleles such as the I and F mutations.

Upon reviewing the European allele frequencies for the Z and S mutations one is compelled to ask the obvious question: why are these two mutations so common? Could there possibly be an advantage to having one of these mutations? This would then provide the selective pressure to retain these mutations. An intriguing theory put forward by David Lomas in an effort to explain the high prevalence of AATD in European populations surmised that the Z and S mutations conferred a survival advantage on heterozygotes in the pre-antibiotic era

(Lomas 2006). The hypothesis describes how Z and S mutations could favour the generation of polymers at sites of inflammation. Polymers of Z and S could then help focus and amplify the host inflammatory response and help eradicate invading infectious organisms. Heat is known to induce Z and S polymer formation and AAT can be produced locally in the gut and the lung, the most common portals for invading pathogens. Febrile episodes in the infected individual could generate large quantities of polymer at the site of infection and recruit protective neutrophils. Thus, the pro-inflammatory response driven by Z polymers was probably hugely advantageous to a population living in the preantibiotic era. However, in the modern era increased life span and cigarette smoking have transformed a previously protective allele into a harmful one. In support of this theory polymers of Z AAT protein isolated from lung lavage have been shown to act as neutrophil chemoattractants and were found localised with neutrophils (Mulgrew et al. 2004; Parmar et al. 2002; Mahadeva et al. 2005). Furthermore, increased neutrophil numbers and IL-8 concentrations have been demonstrated in sputum from MZ heterozygotes (Malerba et al. 2006). It is also possible that the intermediate levels of AAT in MZ and MS individuals are associated with more exuberant protease activity and this allows for faster and more potent protease-dependent killing of harmful pathogens. Comprehensive studies in MZ and MS heterozygotes are required to provide additional evidence for this attractive theory.

2.2.4 Treatment and management

Although there is still no cure for AATD the accurate and early diagnosis of the condition remains imperative for improved health outcomes in AATD individuals. As soon as a diagnosis is established, AATD individuals can benefit from behavioural changes, careful management, and more targeted therapies. Effective management is guided by smoking cessation, vaccine administration and aggressive management of respiratory exacerbations (American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). Augmentation therapy, while prescribed in many countries, remains to be conclusively proven to benefit AATD patients (McCarthy and Dimitrov 2010). Specialised referral centres for AATD are urgently needed to ensure the best care and management of AATD patients. The national referral centre for AATD in Ireland has a rapid access weekly AATD clinic for newly-diagnosed ZZ, SZ, and MZ individuals. The clinic is coordinated by a dedicated clinical research nurse and AATD individuals are seen by a multidisciplinary team of doctors, nurses, and physiotherapists with international best practice standards of care followed. Similar models are being followed or implemented in several European countries but not all.

To examine the importance of early diagnosis and mode of ascertainment in AATD we collected spirometry data from 73 ZZ individuals enrolled in the Irish National AATD Registry. After stratification for mode of ascertainment, the mean % predicted FEV_1 (forced expiratory volume in one second) was significantly higher in ZZ subjects diagnosed through family screening, compared to ZZ subjects who were diagnosed because of symptoms (Fig. 5). While impaired lung function would be expected in the symptomatic group, the preserved lung function in the family screened cohort, despite similar age and smoking history, highlights the importance of family screening as a tool for early detection and possible prevention of COPD in ZZ individuals. The findings from the Irish registry are
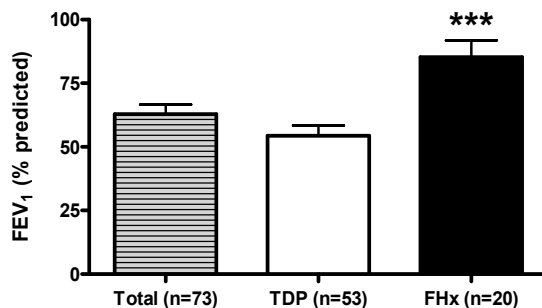
Fig. 5. Mean FEV_1 (% predicted) in ZZ individuals diagnosed by symptomatic screening in a targeted detection programme (TDP) versus those diagnosed via family screening (FHx) enrolled in Irish National AATD Registry (***p < 0.0001, t-test). The mean age of the TDP cohort was 46.1 +/- 1.4 years compared to 41.2 +/- 2.8 years in the family screened cohort, while 77% of the TDP cohort was ever-smoking compared to 65% of the family screened cohort.

supported by a study from the Danish registry which found that non-index ZZ individuals had longer estimated life expectancies when compared to index (symptomatic) cases (Seersholm, Kok-Jensen, and Dirksen 1994). More recently, data from the Swedish registry showed that ZZ individuals identified by family screening had longer median survival times compared to ZZ individuals detected by symptomatic (respiratory and non-respiratory) screening (Tanash et al. 2010).

Many of the early guidelines for AATD screening advocated testing younger COPD patients and this is to the detriment of the larger COPD population of all ages. The age at which manifestations of airway obstruction, pulmonary emphysema, or chronic bronchitis appear in ZZ individuals is highly variable (Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. The Alpha-1-Antitrypsin Deficiency Registry Study Group 1998). While a common presentation of AATD is indeed early onset COPD, a subset of ZZ patients do not develop symptoms until much later in life, particularly if non-smokers (Campos, Alazemi, Zhang, Salathe, et al. 2009). In fact, among ZZ never-smokers the risk of liver disease increases with age (Tanash et al. 2008; Willson, Seow, and Zimmerman 2004). Numerous case reports have described AATD in elderly individuals with COPD who were lifelong never smokers (Jack and Evans 1991). Taken together, it is clear that screening for AATD should be performed in all patients with COPD regardless of advanced age or smoking history, especially as failure to do so has clinical repercussions for undiagnosed family members.

2.2.4.1 Smoking cessation & occupational exposures

While risk factors such as male gender and asthma can contribute to lung disease risk (Demeo et al. 2007), cigarette smoke is by far the single most important risk factor for the development of COPD in AATD patients (Janoff and Carp 1977; Mayer et al. 2006; Seersholm and Kok-Jensen 1995). It has been shown that smoking can reduce the life expectancy of a ZZ patient by up to 25 years (Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. The Alpha-1-Antitrypsin Deficiency Registry Study Group 1998). All AATD patients, including ZZ, SZ, and MZ phenotypes, need to be

educated about the harmful effects of cigarette smoke. Smoking cessation and the avoidance of occupational and environmental exposures (for example particulate matter, chemical vapours, and agricultural dusts) is paramount in AATD patient education (ATS/ERS guidelines, 2003). AATD patients without apparent lung disease should also be encouraged to quit smoking as this cohort offers the most realistic chance of delaying or in some cases preventing the development of COPD. Another important benefit in diagnosing a COPD patient with AATD is that he/she is twice as likely to attempt to quit smoking compared to an AAT-replete, smoking-related COPD patient (Carpenter et al. 2007). Carpenter *et al.* demonstrated that knowledge of AATD motivates smokers towards cessation when compared with COPD patients.

To illustrate the effect of smoking on lung function in AATD patients we collected spirometry data from 70 ZZ patients enrolled in the Irish National AATD Registry and stratified the group according to smoking history. The contribution of occupational exposures was not taken into account; nevertheless, the mean FEV_1 (% predicted) was significantly higher in ZZ subjects who never smoked compared to ZZ subjects who ever smoked, with no difference in mean age between groups (Fig. 6). This clearly demonstrates the major role of tobacco consumption in the pathogenesis of COPD in ZZ individuals.
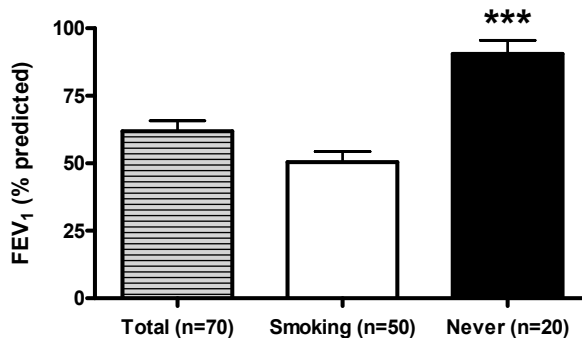

Fig. 6. Mean FEV_1 (% predicted) in ever-smoking versus never-smoking ZZ individuals enrolled in Irish National AATD Registry (***$p < 0.0001$, t-test). Mean age of ever-smoking cohort (50.9 +/- 1.4 years), mean age of never-smoking cohort (50.0 +/- 2.9 years).

However, even in the absence of significant smoking history there exists a significant risk for COPD. The first study to investigate a non-smoking ZZ cohort observed marked variability in both clinical course and lung function decline (Black and Kueppers 1978). Another US study showed that exposure to second-hand tobacco smoke in childhood can accelerate the onset of symptoms in ZZ AATD individuals (Mayer et al. 2006). A study from the Swedish registry demonstrated that, while non-smoking ZZ individuals may not develop COPD until later in life, this cohort still displays a decline in lung function (FEV_1) with age, especially after the age of 50 (Piitulainen, Tornling, and Eriksson 1997). A follow up study by the same group found that an agricultural occupation was associated with decreased lung function in non-smoking ZZ individuals (Piitulainen, Tornling, and Eriksson 1998). Passive smoking was associated with an increased frequency of chronic bronchitis, but not with impaired lung function. Anecdotally, several of the ZZ individuals with COPD attending our clinic are farmers, specifically poultry and grain. A number of the other

occupations reported in our ZZ COPD cohort include welders, chemical factory workers, painters, and firemen.

In the Irish health system there are dedicated smoking cessation officers both in the hospital setting and in the community, as well as another grade of health promotion officers, whose remit also includes smoking cessation. Exposure to second-hand cigarette smoke is also harmful to the lungs of AATD patients and the most important development in the area of lung health in Europe since the introduction of smokeless fuels has been the adoption in many countries of the ban on smoking in the workplace. After many decades of anti-smoking legislation and campaigning the Irish government implemented a law banning smoking in the workplace in 2004 (McElvaney 2004). This was the first law of its kind in Europe and paved the way for a host of other European countries to introduce similar laws (Rada 2010). The positive impact on Europe's lung health may not be significantly visible for several years, but already cigarette consumption in Ireland has dropped to 23.6% (Office of Tobacco Control, www.otc.ie) and this can only be a good thing for AATD individuals.

2.2.4.2 Vaccinations

Influenza and pneumococcal vaccinations are recommended for all individuals with AATD (American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). Campos et al., (2007) investigated the practice of vaccinations and respiratory outcomes in AATD individuals in the United States and showed over 80% of AATD individuals had received adequate influenza and pneumococcal vaccinations during the influenza season (Campos et al. 2008). However, there was no significant difference in severity or rate of exacerbations between vaccinated and unvaccinated individuals but the authors concluded that the vaccinated group may represent 'sicker' AATD individuals. Influenza and pneumococcal vaccinations in COPD patients are documented as being effective and beneficial for patients (Halpin 2004; Fromer and Cooper 2008) and therefore vaccinations remain highly recommended for AATD individuals (American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). Further studies are necessary to ascertain the benefits of vaccinations for AATD individuals, especially whether protection is afforded during the influenza season.

2.2.4.3 Exacerbation management

Exposure to bacterial and viral infections can potentially result in a respiratory exacerbation. Symptoms typically include increased dyspnoea, cough, and production of sputum (Hoogendoorn et al. 2010). Aggressive treatment of infections is recommended with antibiotics and specific treatments as symptomatically required as per ATS/ERS guidelines. This is particularly important as exacerbations adversely affect COPD patients and frequent exacerbations have been shown to be related to worsening health-related quality of life (HRQoL). Predictors for frequent exacerbations in COPD patients include symptoms of wheeze, cough and sputum production (Seemungal et al. 1998). Needham and Stockley (2005) investigated health status in AATD individuals over 12 months and recorded exacerbations, lung function and HRQoL. The authors concluded exacerbations occur commonly in AATD patients and correlate to worse health status. Exacerbations were associated with a decline in the gas transfer of the lung for carbon monoxide over time

(DLCO), but not FEV_1 (Needham and Stockley 2005). Interestingly, a study investigated exacerbation frequency in AATD patients with COPD who were receiving augmentation therapy and found subjects with frequent exacerbations had the worst baseline HRQoL scores, as well as more physician visits and hospitalizations. Unfortunately, AATD patients not receiving augmentation therapy were not included for comparison (Campos, Alazemi, Zhang, Wanner, Salathe, et al. 2009).

A recent longitudinal study by Campos et al., (2009) undertaken in the United States, evaluated the effectiveness of a disease management and prevention program for AATD individuals, involving 905 individuals, over a 2 year period. The program comprised of written educational material for self-study and individualised treatment plans for exacerbations. This study illustrated improved patient compliance in the use of bronchodilators, oxygen therapy, and steroids during exacerbations. The management program significantly reduced medical visits and showed a considerably slower deterioration of HRQoL during an exacerbation (Campos, Alazemi, Zhang, Wanner, and Sandhaus 2009). A follow-up study would be beneficial by providing additional evidence to evaluate the long-term benefits of an AATD disease management program.

2.2.4.4 Replacement therapy

Replacement therapy is a specific therapy for AATD, and the therapy comprises of intravenous administration of AAT derived from human plasma (Stoller and Aboussouan 2004). At present, this treatment is available in a number of European countries and the United States (Chapman et al. 2009). Some AATD individuals may be candidates for AAT replacement therapy; however, the efficacy of this treatment remains controversial (McCarthy and Dimitrov 2010). Uncertainty persists concerning the therapy's effectiveness and ongoing randomised clinical trials are being performed to definitively assess the efficacy of the treatment. Previous trials have been under-powered and have mostly shown only biochemical efficacy with AAT levels restored to above the putative threshold in the blood and lung. There is some evidence that augmentation therapy can slow lung function decline in patients with AAT deficiency, however, patients with moderate obstruction are most likely to benefit (Modrykamien and Stoller 2009). The therapy comprises of weekly or fortnightly intravenous infusion of an AAT preparation that augments existing levels of circulating AAT in the blood.

3. Alpha-1 antitrypsin deficiency (AATD) and COPD

3.1 COPD

The World Health Organisation definition of chronic obstructive pulmonary disease (COPD) is a lung disease characterized by chronic obstruction of lung airflow that interferes with normal breathing and is not fully reversible. The more familiar terms 'chronic bronchitis' and 'emphysema' are still in common use, but are to be included within the umbrella term COPD. COPD is not simply a "smoker's cough" but an under-diagnosed, life-threatening lung disease. A diagnosis of COPD should be considered in any patient who has symptoms of cough, sputum production, or dyspnea, and/or a history of exposure to risk factors for the disease. Diagnosis is confirmed by spirometry, however, even with the ready availability of a simple test, COPD is largely under-diagnosed (Mannino and Braman 2007). Despite being a very common disease which affects 5% of the US population and the fourth

leading cause of death in the United States (Eisner et al. 2010), COPD often is a silent and unrecognised disease, particularly in its early phases (Mannino et al. 2000). Globally, COPD is a growing cause of mortality and will become the third biggest killer by 2020 according to the WHO (WHO 2004).

3.2 What we have learned about COPD from AATD

In the mid-1960s there were two major discoveries that led to an exponential increase in our knowledge of COPD and the development of the proteolytic hypothesis of lung disease. These were the discovery of AATD and its association with COPD, and the induction of emphysema by intratracheal instillation of a protease (Gross et al. 1965). While numerous different elastases were subsequently shown to cause emphysema in the same model, none of the elastases known at that time had access to the human lung. This changed in 1968 when a potent elastase was discovered within human neutrophilic leukocytes, the primary acute inflammatory white blood cell in the body (Janoff and Scherer 1968). Called neutrophil elastase or NE, it was found to be exquisitely sensitive to inhibition by AAT. It was then discovered that certain products of cigarette smoke were able to destroy the anti-elastase properties of AAT (Johnson and Travis 1979). While AAT is an excellent inhibitor of NE, the Met358 amino acid at its active site is easily oxidised by cigarette smoke and oxidants released by immune cells (Carp et al. 1982; Hubbard et al. 1987). On foot of these revelations, it was proposed in the 1970s that all COPD could be due, at least in part, to a deficiency of AAT (Gadek, Fells, and Crystal 1979). In the majority of individuals who develop COPD due to smoking, the deficiency is functional and due to the inactivation of AAT by cigarette smoke and the influx of inflammatory cells. In individuals with AATD, the deficiency is genetic.

3.3 The ZZ phenotype as a genetic risk factor for COPD

While smoking-related COPD is acquired, AATD is a form of inherited COPD and is responsible for approximately 1 – 3 % of COPD cases. A US study in 1986 investigated 965 COPD patients and found 1.9% were ZZ and over 8% were MZ (Lieberman, Winter, and Sastre 1986). Another US study investigated 969 patients with diagnosed with emphysema, asthma, or chronic bronchitis and found 1 ZZ case in every 31 samples, which is a case detection rate of over 3% (Brantly M 2003). Moreover, the contribution of SERPINA1 heterozygosity to COPD, while controversial, may account for over 10% of COPD cases if one includes ZZ, SZ and MZ phenotypes (Carroll et al. 2011). The classic pulmonary presentation of lung disease in AATD is severe, early onset panacinar emphysema with a basilar predominance in adults (Gishen et al. 1982). Evaluation of the lungs in ZZ individuals often shows diffuse destruction of the alveoli, first in the lower lung zones, and eventually throughout the entire lungs (Parr et al. 2004). This contrasts with the classic pattern of emphysema observed in smoking-related COPD, which is centrilobular (centriacinar) (Kim et al. 1991). However, emphysema in ZZ individuals may also occur in a diffuse distribution or predominantly in the upper lobes (Parr et al. 2004). Bronchiectasis, with or without accompanying emphysema, is less frequent (Parr et al. 2007). The most prominent early symptom is dyspnea, particularly upon exercise (McElvaney et al. 1997).

COPD is characterized by neutrophil-dominated airway inflammation and elevated protease levels in the lung (Abboud and Vimalanathan 2008). The main physiological role of AAT is to protect fragile alveolar lung tissue from attack by proteases, in particular

neutrophil elastase. NE is a powerful protease and can degrade most protein components of the extracellular matrix (Taggart et al. 2005), several complement proteins and immunoglobulins (Tosi, Zakem, and Berger 1990; Fick et al. 1984), antimicrobial proteins (Britigan et al. 1993; Hirche et al. 2004), and other antiproteases such as secretory leucoprotease inhibitor (SLPI) and elafin (Weldon et al. 2009; Guyot et al. 2008). NE can also induce mucin production and inflammatory gene expression in the lung (Fischer and Voynow 2002; Kohri, Ueki, and Nadel 2002; Carroll et al. 2005). In addition, there is the prospect that NE is situated at the apex of a hierarchical tree of cysteine and metalloproteases, acting as a master regulator of several classes of tissue-degrading proteases (Geraghty et al. 2007). The role of AAT in regulating NE activity *in vivo* is underscored by the fact that inhaled AAT therapy reduced MMP-2 and cathepsin B activity in lavage fluid from ZZ patients (Geraghty et al. 2008).

The traditional protease-antiprotease imbalance theory which explains COPD in ZZ individuals by a loss of function mechanism, while certainly attractive, is not the only explanation for the development of COPD. There are a host of gain of function effects caused by mutations within the SERPINA1 gene discussed in more detail elsewhere (Carroll, McElvaney, and Greene 2010; Greene and McElvaney 2010; Ekeowa, Marciniak, and Lomas 2011). Evidence is mounting to suggest other pathways contribute to tissue injury and inflammation. For example, rogue Z AAT protein can form polymers, and these polymers are present in the epithelial lining fluid of the lung. Polymers of Z AAT made by lung cells or reaching the lungs through the blood can cause the local release of chemokines and the recruitment of immune cells to the lung, contributing to the neutrophilic inflammation characteristic of COPD (Parmar et al. 2002; Mulgrew et al. 2004; Mahadeva et al. 2005). In addition, the expression of Z AAT in immune cells can lead to a more exuberant immune response. Monocytes from asymptomatic ZZ individuals with preserved lung function produced more pro-inflammatory cytokines and chemokines than MM individuals, and this inflammatory phenotype may explain some of the predisposition to COPD (Carroll et al. 2010).

Additional pathways leading to tissue injury highlighted recently include a role for AAT in apoptosis and in the regulation of chemotaxis. Wild-type AAT protein prevents lung alveolar endothelial cell apoptosis, possibly by inhibiting caspase-3 (Petrache, Fijalkowska, Medler, et al. 2006) and reducing oxidative stress (Petrache, Fijalkowska, Zhen, et al. 2006). These pro-survival benefits are lacking in ZZ individuals and could favour structural cell apoptosis and contribute to the development of emphysematous changes, particularly as the COPD lung is an environment with high levels of oxidative stress (Yao and Rahman 2011). A novel anti-inflammatory role for AAT as a "brake" on immune cell chemotaxis was also described. Wild-type AAT was shown to regulate neutrophil chemotaxis by both binding the chemokine IL-8 and preventing shedding of the immune receptor FcγRIIIb (Bergin et al. 2010).

The data accumulated unequivocally demonstrates that the ZZ phenotype is a major risk factor for COPD and this is thought to be mediated by at least four pathological mechanisms:

1. Increased protease activity in the lung,
2. Polymer formation locally,
3. Loss of anti-apoptotic properties of AAT,
4. Loss of anti-inflammatory properties of AAT.

3.4 The SZ phenotype as a genetic risk factor for COPD

Historically there has been a widespread acceptance that the SZ genotype confers increased susceptibility to COPD, particularly in smokers. This could explain why the susceptibility of SZ subjects to COPD has not been the subject of as many studies compared to MZ subjects. In addition, the typical SZ serum AAT level of approximately 11 µM is also deemed the putative protective threshold above which there is presumed to be no increased risk for emphysema in individuals with AATD and it is this level at which augmentation therapy levels are aimed (Wewers et al. 1987). Unfortunately, as a result there have been few studies aimed at assessing COPD risk in SZ individuals and most of these have been underpowered. The first study examined 25 cases, 14 of whom were index cases and concluded the SZ phenotype was of much less importance than the ZZ type in the development of emphysema (Hutchison, Tobin, and Cook 1983). Another study concluded that only a small percentage of SZ individuals are at increased risk of developing emphysema and that in non-smoking individuals the SZ phenotype conferred little or no added risk of developing COPD. However, it was noted that cigarette smoking correlated more strongly with airflow obstruction in SZ rather than ZZ subjects. Again this was a relatively small study of 59 individuals with no specific distinction between index and non-index subjects (Turino et al. 1996). In 1998 a Danish group investigated a cohort of 94 SZ individuals on the Danish AATD Registry and came to the same conclusion that a small proportion of SZ individuals are at increased risk of emphysema (Seersholm and Kok-Jensen 1998). A meta-analysis in 2005 examining COPD risk in the SZ group sought to shed some light on the issue and calculated that there was a three-fold elevated risk of COPD (Dahl et al. 2005). Unfortunately, due to the limited number of subjects with accurate smoking information, it was not possible to calculate separate odds ratios for SZ smokers and non-smokers. The most recent study was an audit of SZ individuals on the UK AATD registry. SZ subjects showed less emphysema on CT scans and less abnormal spirometry test results, but equivalent health status impairment compared to matched ZZ subjects (Holme and Stockley 2009). Like the MZ genotype, attempts to explain the risk of COPD in SZ subjects have stopped at the decreased AAT levels, and other pathological mechanisms have not been explored.

3.5 The MZ phenotype as a genetic risk factor for COPD

It is well established that MZ heterozygotes have moderately reduced serum levels of AAT, but whether they have an increased risk of COPD remains an area of some controversy. Over the last 40 years, over 100 studies have attempted to assess the risk of lung disease in MZ individuals with discordant and contentious results. A meta-analysis of 22 of these studies was published in 2004 (Hersh et al. 2004). Six of the 16 studies examining the categorical outcome of obstructive lung disease found significantly increased odds ratios (OR) for COPD in MZ heterozygotes compared to MM individuals. In nine other studies, the OR was increased, but not significantly. The individual study ORs ranged from 0.15 to 16.78. In summary, the study found that the OR for COPD in MZ compared to MM individuals was elevated at 2.31 (95% CI 1.60 to 3.35). Since this meta-analysis another US study has shown that MZ individuals exhibit accelerated decline in diffusing capacity of the lung for carbon monoxide (DLCO) in a large prospective population-based study of 1,075 individuals (Silva et al. 2008). More recently, a 2010 study

demonstrated that MZ heterozygosity was associated with airflow obstruction in two large populations (Sorheim et al. 2010). In general, studies comparing COPD cases with healthy controls have found an excess of MZ individuals among COPD cases, but many studies comparing FEV_1 (% predicted) in MZ and MM subjects from population-based samples have not found significant differences. There are several factors that undermine many of these conclusions. Firstly, a lack of correction for active and passive cigarette smoking exists in many studies. Secondly, the use of spirometry in defining lung disease is flawed, as subjects with normal spirometry values can have evidence of emphysema on high-resolution CT (Spaggiari et al. 2005). However, it is clear that the weight of evidence is now in favour of a risk of COPD in MZ individuals, but explanations of this risk are limited to the decreased antiprotease levels in subjects, and have not taken into account alternative mechanisms such as Z polymer generation. In the setting of disease management, an individual with smoking-related COPD who is informed of a diagnosis of MZ AATD may be further motivated towards smoking cessation (Carpenter et al. 2007).

3.6 Other AATD phenotypes as genetic risk factors for COPD

Early studies that examined the relatively common MS phenotype found no increase in COPD risk but an increase in bronchial hyperreactivity among MS individuals (Townley et al. 1990) but this was not replicated in a larger study (Miravitlles et al. 2002). In a recent meta-analysis of case-control and cross-sectional studies examining COPD risk in MS individuals a small but significantly increased risk was found (Dahl et al. 2005). However, after correction for smoking the MS phenotype was not associated with elevated risk for COPD. Moreover, studies that measured pulmonary function did not find a difference between MS and MM individuals. Another study did attempt to address the risk of COPD in SS individuals but found no increased risk of obstructive lung disease and was limited by small sample size (McGee et al. 2010). The effect of the I and F mutations on the AAT molecule has been described earlier, but to date any mention of COPD risk associated with these mutations is limited to case reports describing compound heterozygotes (Kelly et al. 1989; Baur and Bencze 1987).

3.7 Conclusion

It is well established that ZZ individuals have a high risk of developing COPD. However, MZ and SZ individuals also have significantly reduced levels of AAT, and are at risk of developing COPD. Anecdotally, a significant number of MZ and SZ individuals (both smokers and non-smokers) from our AATD clinic have severe COPD at a young age. The risk of COPD in heterozygotes has traditionally been explained by the weakening of the antiprotease shield in the lung. However, we know the Z mutation confers harmful gain of function properties on the AAT protein. While the ZZ genotype is relatively well-studied, there is little information regarding MZ, SZ, and other less common genotypes and to date there have been no investigations into the functional consequences of AATD heterozygosity. This is a vital clinical and public health question, as there are predicted to be over 6.6 million MZ and 230,000 SZ individuals in the US alone (de Serres, Blanco, and Fernandez-Bustillo 2010) and the total economic costs of COPD in the US were estimated to be almost $50 billion in 2010 (National Heart 2009). From a basic research perspective, a careful

examination of AATD heterozygosity may lead to a new appreciation of this understudied area and the development of new therapies.

4. Testing for alpha-1 antitrypsin deficiency (AATD)

4.1 Who should be tested?

Guidelines issued by both the World Health Organisation and the American Thoracic Society/European Respiratory Society (ATS/ERS) recommend the establishment of targeted screening programmes for the detection of patients with AATD (Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting 1997; American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). The biggest problem in the area of AATD is under-diagnosis with most cases misdiagnosed as COPD or non-responsive asthma. As a result, long delays between presentation of first symptoms and correct diagnosis are commonplace and prevent optimal management of the condition, despite education and awareness efforts (Stoller et al. 2005; Campos et al. 2005; Kohnlein, Janciauskiene, and Welte 2010). Compared to population-based studies, which are difficult and expensive to perform on a large scale, targeted detection programmes offer a much higher rate of AATD detection, are easier to perform, and are more cost-effective. However, as they target symptomatic groups the possibility of missing asymptomatic individuals remains. For this reason, comprehensive screening of family members of known AATD individuals is crucial as it offers the most realistic prospect of detecting asymptomatic relatives (Hogarth and Rachelefsky 2008). In the Irish targeted detection programme first-degree relatives of not only ZZ, but SZ and MZ individuals are recommended for testing.

Data from several countries suggests that less than 10% of individuals with severe AATD have been recognised clinically (Aboussouan and Stoller 2009), and improving detection rates is the most urgent issue in the coming years. Several barriers to testing for AATD in

ATS/ERS Recommendations for Diagnostic Testing
Adults with symptomatic emphysema or COPD
Adults with asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators
Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g. cigarette smoking, occupational exposure)
Adults with necrotising panniculitis
Siblings of individuals with AATD
Individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly

Table 2. ATS/ERS recommendations for diagnostic testing for AATD (type A recommendations).

the COPD population exist, including a fear of genetic discrimination, financial concerns, and privacy concerns (Stoller et al. 2007). Fears of genetic discrimination have been allayed in recent years with preventative legislation enacted in several countries, including Ireland and the US. Barriers to testing among physicians include lack of awareness and knowledge of AATD, lack of access to testing methods, and testing fatigue among physicians who do not encounter AATD initially and give up testing. An element of therapeutic nihilism can also exist, with the mistaken belief that identifying AATD in a COPD patient offers no immediate clinical benefit. Initiatives to increase detection rates might include automatic physician alerts suggesting AATD testing on pulmonary function test reports of patients with fixed airflow obstruction (Rahaghi et al. 2009), better medical and patient education in the area of AATD (Fromer 2010), and a red flag to recommend testing for AATD on laboratory reports of patient with low levels of AAT. The strategy of electronic prompting offers the greatest potential, and has been trialled in several regional hospital laboratories in Ireland to great effect.
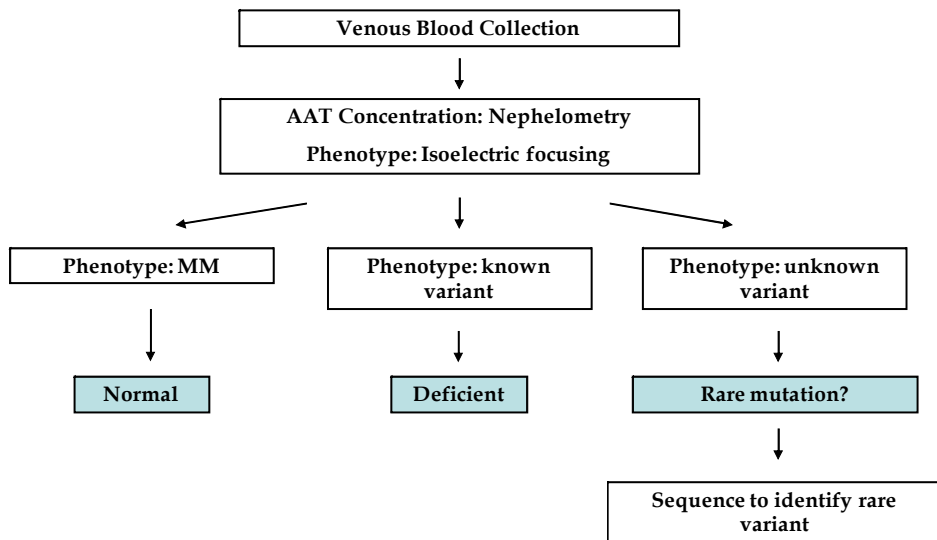
Fig. 7. Diagnostic algorithm for testing of whole blood utilised in the Irish National AATD Targeted Detection Programme (TDP). All IEF results are correlated to the quantification of serum AAT.

The advent of finger-prick tests using dried blood spots (DBS) as a source of DNA has allowed home testing for AATD, with easier transportation of samples to the laboratory (Costa et al. 2000). This method of testing eliminates the fear of needles for the patient, and is also cheaper as the test does not require a visit to a general practitioner. Identification of a deficient variant should be confirmed with serum or plasma AAT quantification, as genotyping of DBS sample can miss rarer alleles such as Mmalton (Rodriguez-Frias et al. 2011). For this reason, finger-prick kits are used in the Irish detection programme only for screening family members of index cases who possess Z or S alleles, with whole blood preferred as this allows identification of rare AAT variants. Several laboratories have developed methods of quantifying AAT from DBS which enhance the diagnostic options

available (Miravitlles et al. 2010). The traditional gold standard for the diagnosis of AATD has been phenotype analysis by isoelectric focusing but there has been a move in the last few years to a combination of genotyping and quantification (Snyder et al. 2006). Ultimately, while there are pros and cons to both methods of sampling, the decision to collect whole blood or DBS is often guided by cost considerations.

4.2 Quantification of AAT

The World Health Organization has recommended that AAT levels should be measured at least once in all COPD patients and this position was supported by the American Thoracic Society (ATS) and the European Respiratory Society (ERS). The most important consideration when quantifying AAT is the fact that, as an acute phase reactant, AAT can be markedly elevated during infection and inflammation. This is especially relevant if testing COPD patients during an exacerbation. While AAT levels in ZZ individuals are so low that any increase is marginal, circulating AAT levels in heterozygotes (both MZ and SZ) can be "falsely" elevated to levels similar to those observed in MM individuals (Fig. 8). A pronounced acute phase response in many individuals is observed in the MM, MS, and MZ groups; however, the acute phase response in SS, SZ, and ZZ groups is blunted. For this reason, quantification of AAT is no substitute for phenotype or genotype analysis, which is not influenced by the acute phase.
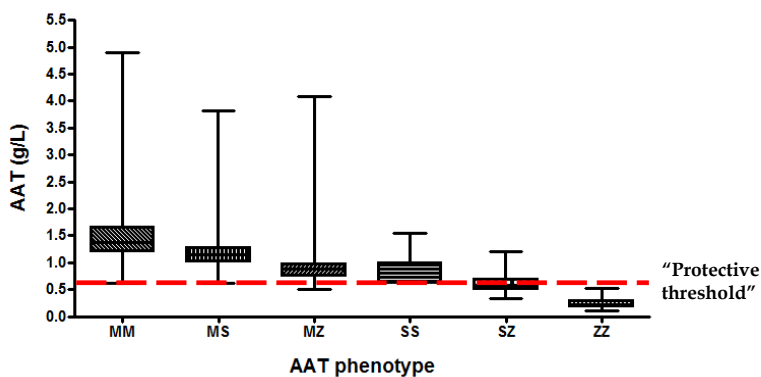

Fig. 8. AAT levels among the various phenotype classes identified in the Irish National AATD Targeted Detection Programme (TDP).

AAT levels are routinely measured by immunoassay techniques such as nephelometry and turbidimetry, or less commonly by radial immunodiffusion (RID) (Viedma et al. 1986). These methods are based on the use of a specific antibody which binds the AAT in a serum sample. Discrepancies can exist when comparing these methods for serum AAT quantification. For example, nephelometric methods can overestimate AAT concentrations due to haemoglobin or lipid interference, while RID-based methods have been shown to overestimate AAT concentrations by as much as 35 – 40% (Brantly et al. 1991) and are less precise than nephelometric methods with higher coefficients of variation (Alexander 1980). Moreover, the lower sensitivity inherent to the RID method because of the high lower limit of detection (0.33 g/L) becomes a factor when testing severely deficient ZZ individuals, as the majority have AAT concentrations < 0.33 g/L.

Phenotype	N	Mean AAT (g/L)	AAT range (g/L)
MM	3621	1.49 +/- 0.01	0.62 – 4.90
MS	568	1.21 +/- 0.02	0.62 – 3.82
MZ	802	0.89 +/- 0.01	0.50 – 4.08
SS	29	0.86 +/- 0.04	0.62 – 1.54
SZ	87	0.61 +/- 0.02	0.33 – 1.20
ZZ	90	0.24 +/- 0.01	0.11 – 0.52

Table 3. AAT phenotypes and serum AAT concentrations analysed as part of the Irish national targeted detection programme (TDP). Some unusually low AAT concentrations in the MM, MS, and MZ groups were measured in patients with chronic liver disease. Data presented as mean AAT (g/L) +/- standard error of the mean (SEM).

An important consideration in optimising screening programmes for AATD is what cut-off level of AAT is suitable to adopt, below which samples should be investigated further by phenotype or genotype analysis. The choice of cut-off level has obvious financial implications and can cause unnecessary anxiety in a patient if inappropriate follow-up testing is performed. Moreover, cut-off values do not apply when testing family members of known AATD individuals or when testing paediatric cases of liver disease. Phenotype or genotype analysis should be performed in these cases regardless of AAT concentration. This can depend on whether the screening programme aims to detect severely deficient individuals (for example ZZ, Z/Null, Z/Mmalton), or if the aim is to also detect MZ heterozygotes. For example, a cut-off of 1.0 g/L may confidently detect 100% of severely deficient AATD cases, but may miss some SZ cases and large percentage of MZ cases (Kok, Willems, and Drenth 2010). A Swiss-Italian study has shown that MZ individuals with a C-reactive protein (CRP) level of > 0.8 g/L had higher mean AAT concentrations than MZ individuals with lower CRP levels, reflecting the acute phase nature of AAT production (Zorzetto et al. 2008). The cautionary note in using CRP levels to correct for systemic inflammation and an acute phase response when measuring AAT is that CRP is also liver-derived. Thus, in patients with chronic liver disease the ability of the liver to produce either AAT or CRP, or both, may be severely impaired. The choice of cut-off for AAT will reflect the goals of the screening programme and depends on the cost of extra testing and budgetary constraints. If financially permitted, we would advocate the phenotypic or genotypic analysis of all COPD patients.

4.3 Phenotyping of AAT

In the manner of Laurell and Eriksson, it is possible to detect ZZ individuals by careful visual inspection of electrophoretic patterns on routine serum protein electrophoresis, particularly in ZZ subjects where the absence of the alpha-1 globulin band is so striking (Malfait, Gorus, and Sevens 1985). However, this diagnostic method is not guaranteed to detect all ZZ cases, and will not detect SZ and MZ phenotypes with any confidence (Slev et al. 2008). For this reason, the gold standard for the diagnosis of AATD is isoelectric focusing, which is based upon the isoelectric point of the AAT protein and separates the various isoforms of AAT based on their migration in a specific pH gradient. Each isoform migrates to the position within the pH gradient where the overall charge of the molecule is zero. Qualitative detection and characterisation of AAT variants is carried out in our laboratories by isoelectric focusing using the Hydrasys electrophoresis platform (Sebia) and the

Hydragel 18 A1AT isofocusing kit (Zerimech et al. 2008). This isoelectric focusing (IEF) method on agarose gel has an added immunofixation step which utilises a specific antibody to AAT, rendering it superior to traditional IEF techniques, and improving the resolution and reproducibility. The AAT phenotype is determined by visual inspection by at least two independent observers and by comparison with reference standards. There are some unusual cases where a phenotype analysis will not lead to correct diagnosis. M/Null, S/Null, and Z/Null individuals will appear as MM, SS, ZZ phenotypes, respectively, although genotyping will not detect Null mutations either. Another example is of a ZZ individual who has received a liver transplant (Hackbarth et al. 2010). This would result in an MM phenotype on serum analysis by IEF. The transplant recipient would have normal AAT levels as a result of the donor liver (presumably MM) but any offspring would still inherit the Z mutation.
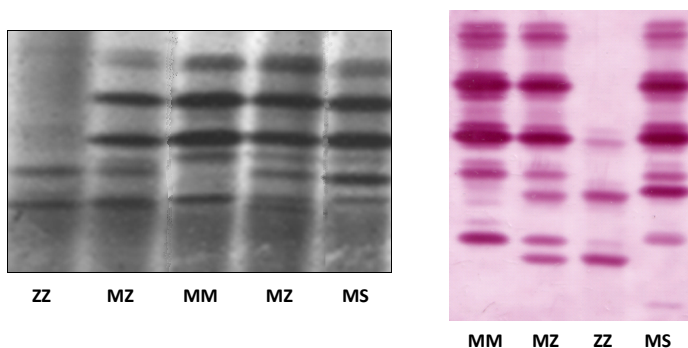
Fig. 9. Technique of isoelectric focusing by polyacrylamide gel electrophoresis (left) compared to isoelectric focusing by agarose gel electrophoresis followed by immunofixation (right).

A novel method for the simultaneous quantification of AAT and identification of the Z and S mutations in a single sample has been recently described which uses liquid chromatography/tandem mass spectrometry (Chen et al. 2011). This has the potential to combine quantification and phenotyping in a single step but a feasibility and cost analysis study is required, and rare AAT mutations are not detected.

4.4 Genotyping of AAT

Genotyping assays are typically performed using PCR-based restriction fragment length polymorphism (RFLP) analysis or by melting curve analysis on real-time PCR instruments with specific primers and probes designed for the Z and S mutations (Ferrarotti et al. 2004; Rodriguez et al. 2002). RFLP methods, although cheaper, are time-consuming and have been superseded by the faster and more efficient melting curve methods (Aslanidis, Nauck, and Schmitz 1999; Bartels et al. 2009).

The advantage of genotyping assays is that they allow the rapid screening of DNA collected by dried blood spots and are not as prone to errors in interpretation as the IEF method. The inherent limitation of the genotyping assay is that most laboratories include only primers for the deficient mutation. This means that in an assay for the Z mutation, a rare mutation will

be mistakenly classified as M, unless specific primers for M AAT are used. This rare mutation should of course be termed "non-Z" but this important distinction is not always made. To avoid any errors in diagnosis, any genotype result should be correlated to the AAT concentration. For example, a Null/Z individual will be classified MZ on a typical genotyping assay, however, consideration of the AAT concentration in this individual should prompt further investigation by sequencing.
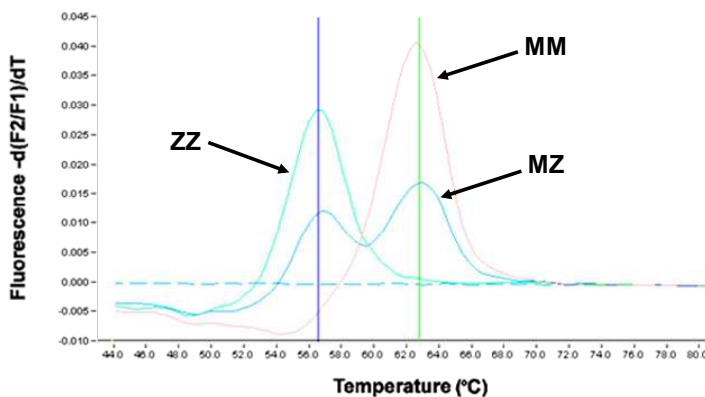

Fig. 10. Genotyping assay for the Z mutation by melting curve analysis on a real-time PCR system (Roche LightCycler 480).

5. Conclusion

The fact that cigarette smoking is often a coincident historical finding in the assessment of COPD has probably contributed to the remarkable global under-diagnosis of AATD. For example, of the 2,000 estimated ZZ individuals in Ireland, only 200 or 10% have been diagnosed (unpublished data). Unfortunately, COPD in a patient with a history of cigarette smoking is often assumed to be purely environmental and testing for AATD is not considered. This is one of a host of misconceptions that have surrounded AATD and prevented optimal management of AATD patients (Stoller and Aboussouan 2009). We strongly believe that increased testing for AATD is desperately needed in the COPD patient community, and stress once again the clinical guidelines that all patients with COPD should be screened for AATD.

The model for COPD diagnosis, assessment and management must include testing for AATD as one of the first steps. Unfortunately, AATD is often relegated to a footnote in many clinical guidelines for COPD. A summary of the ATS and ERS document outlining standards for the diagnosis and treatment of patients with COPD published in 2004 mentioned AATD once, stating that "patients presenting with airflow limitation at a relatively early age (4th or 5th decade) and particularly those with a family history of COPD should be tested for alpha-1 antitrypsin deficiency" (Celli and MacNee 2004). This narrow definition is damaging to efforts at identifying all cases of AATD. Large variability exists in the clinical course of lung disease in AATD and therefore all COPD patients should be tested for AATD, regardless of age or smoking history. Any management strategy for COPD must include testing for AATD. The under-diagnosis of AATD in the COPD population is a situation that cannot be allowed to continue.
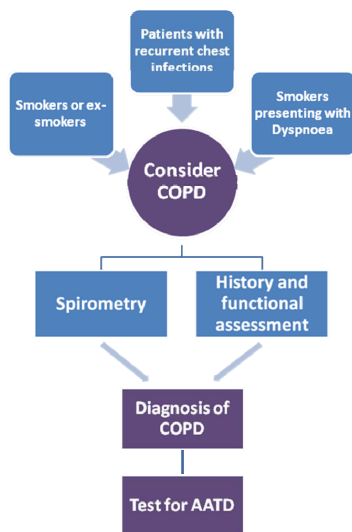
Fig. 11. Model for diagnosis of COPD and AATD.

6. Acknowledgement

7. References

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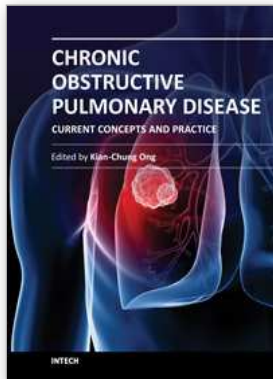
Chronic Obstructive Pulmonary Disease - Current Concepts and Practice

Edited by Dr. Kian-Chung Ong

ISBN 978-953-51-0163-5

Hard cover, 474 pages

Publisher InTech

Published online 02, March, 2012

Published in print edition March, 2012

A decade or so ago, many clinicians were described as having an unnecessarily 'nihilistic' view of COPD. This has certainly changed over the years... This open access book on COPD provides a platform for scientists and clinicians from around the world to present their knowledge of the disease and up-to-date scientific findings, and avails the reader to a multitude of topics: from recent discoveries in the basic sciences to state-of-the-art interventions on COPD. Management of patients with COPD challenges the whole gamut of Respiratory Medicine - necessarily pushing frontiers in pulmonary function (and exercise) testing, radiologic imaging, pharmaceuticals, chest physiotherapy, intensive care with respiratory therapy, bronchology and thoracic surgery. In addition, multi-disciplinary inputs from other specialty fields such as cardiology, neuro-psychiatry, geriatric medicine and palliative care are often necessary for the comprehensive management of COPD. The recent progress and a multi-disciplinary approach in dealing with COPD certainly bode well for the future. Nonetheless, the final goal and ultimate outcome is in improving the health status and survival of patients with COPD.

How to reference

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Tomás P. Carroll, Catherine A. O'Connor, Emer P. Reeves and Noel G. McElvaney (2012). Alpha-1 Antitrypsin Deficiency – A Genetic Risk Factor for COPD, Chronic Obstructive Pulmonary Disease - Current Concepts and Practice, Dr. Kian-Chung Ong (Ed.), ISBN: 978-953-51-0163-5, InTech, Available from: http://www.intechopen.com/books/chronic-obstructive-pulmonary-disease-current-concepts-and-practice/alpha-1-antitrypsin-deficiency-a-genetic-risk-factor-for-copd

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